Evaluation of α -Iduronidase in Dried Blood Spots is an Accurate Tool for Mucopolysaccharidosis I Diagnosis

Karen B. Müller,¹ Vanessa G. Pereira,¹ Ana M. Martins,¹ and Vânia D'Almeida^{1,2*}

¹Department of Pediatrics, Universidade Federal de São Paulo, São Paulo, Brazil

²Department of Psychobiology, Universidade Federal de São Paulo, São Paulo, Brazil

Background: Mucopolysaccharidosis type I (MPS I) is caused by a deficiency of the α -L-iduronidase (IDUA), which leads to the accumulation of glycosaminoglycans in lysosomes. MPS I patients present a spectrum ranging from a severe to an attenuated phenotype. Once clinical suspicion is present, diagnosis of MPS I can be performed by enzyme activity determination and/or molecular analysis. The aim of this study was to establish a reference interval value to IDUA activity using a dried blood spots (DBS) assay and to evaluate whether this assay could be a secure tool to diagnose MPS I patients. **Results:** IDUA activity range on HV DBS samples were 1.40–7.78 $\mu\text{mol/l}$

blood/hr. Regarding the validation group, 11 of the 36 individuals clinically suspected of MPS I had the diagnosis confirmed by DBS and reference assay (leukocytes). When we considered the new proposed cutoff value of 1.5 $\mu\text{mol/l}$ blood/hr, the sensitivity, specificity, and predictive values were 100%. **Conclusions:** Our results strongly suggest that the determination of IDUA activity using a DBS assay is a secure tool for MPS I diagnosis. However, it is extremely important to assure that all recommendations for collection, transport, and storage are correctly followed to guarantee the quality of the samples. J. Clin. Lab. Anal. 25:251–254, 2011. © 2011 Wiley-Liss, Inc.

Key words: lysosomal storage disease; enzyme activity; fluorimetric assay; dried blood spots; α -iduronidase; mucopolysaccharidosis type I

INTRODUCTION

Mucopolysaccharidosis type I (MPS I) is an autosomal recessive lysosomal storage disorder caused by a deficiency of the enzyme α -L-iduronidase (IDUA). As a result of this defect, there is an accumulation of two glycosaminoglycans—dermatan and heparan sulphate—in the lysosomal compartment of different cell types throughout the body (1). MPS I patients present a wide spectrum of clinical manifestations that are characterized by progressive multisystemic deterioration, ranging from a severe (Hurler Syndrome) to an attenuated phenotype (Scheie Syndrome) through an intermediate form (Hurler–Scheie Syndrome) (1).

The diagnosis of MPS I patients can be performed by enzyme activity determination and/or molecular analysis of the *IDUA* gene. Once clinical suspicion is present, an early and effective diagnosis is crucial for prognosis and the success of any strategy of therapy. Enzyme activity determination can be performed by fluorimetry, immunocapture, and mass spectrometry in different biological fluids (2–5). However, the difficulty to obtain

high-quality samples and transportation issues are often a limitation for a rapid diagnosis. Recently, a fluorimetric method of IDUA activity determination for MPS I diagnosis using dried blood spots (DBS) on filter paper was published (6,7).

The aim of this study was to establish a reference interval value to IDUA activity by DBS method in a Brazilian sample and to evaluate the accuracy of this assay for MPS I diagnosis.

MATERIALS AND METHODS

A total of 164 Brazilian healthy volunteers (HV) from both genders (69 men and 95 women—18 until 73 years) and who did not show any clinical evidence of chronic

*Correspondence to: Vânia D'Almeida, Rua Napoleão de Barros, 925 – 3rd floor – room 10, Vila Clementino, 04024-002, São Paulo – SP – Brazil. E-mail: vaniadalmeida@uol.com.br

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disease and/or other genetic disease already diagnosed were eligible to compose the control group. Blood (5 ml) was collected in heparin-containing tubes by vessel puncture and DBS were prepared on Whatman® 903 filter paper. Samples collected at the Universidade Federal de São Paulo (UNIFESP) were stored at the same day at 4°C and those that were collected in other locations were sent to the laboratory at a maximal interval of 5 days, where they were stored at 4°C.

The validation group (VG) comprised DBS and leukocyte samples from 36 individuals clinically suspected of MPS I, sent by physicians from 12 different Brazilian States. Only high-quality DBS (assessed by macroscopic appearance and determination of β -galactosidase activity (8,9)) were accepted. Leukocyte samples were isolated from blood in heparin-containing tubes, which were sent to our laboratory along with DBS samples. Those were only included in the study if they had been transported under refrigeration and collected in a maximum of 2 days. The leukocyte isolation method has been described by Skoog and Beck (10), and samples were stored at -20°C until analysis.

Research protocols and consent forms as well as the overall investigation were ethically and scientifically approved by the Medical Research and Ethical Committee of the UNIFESP (0384/05). DBS enzyme activity assay was previously validated by our group based on the method described by Chamoles et al. (6,7,11). Results were expressed as $\mu\text{mol/l blood/h}$. Determination of IDUA activity in leukocytes was performed as the reference test, following the method described by Rome et al. (5). Results were expressed as $\text{nmol/mg protein/hr}$. Protein quantification was performed through a commercial kit (RC DCTM Protein Assay; BIORAD Laboratories, CA).

Cutoff values initially used to discriminate HV from MPS I patients were $1.10 \mu\text{mol/l blood/hr}$ and $0.02 \text{ nmol/mg protein/hr}$ for DBS and leukocyte assays, respectively (5). Data from enzyme activity are expressed as mean $\pm$ standard deviation. Sensitivity, specificity, predictive values, and likelihood ratio of IDUA activity at different cutoff values were determined by the construction of a receiver operator characteristic (ROC) curve including all individuals from VG. All analyses were performed with GraphPad Prism 5 for Windows software (La Jolla, CA).

RESULTS

IDUA activity on DBS samples for the HV group ranged between 1.40 and $7.78 \mu\text{mol/l/hr}$. β -galactosidase activity determination was performed on all samples and results were in accordance with the accepted reference range for this enzyme activity (11).

Assay imprecision was calculated by replicate analysis of DBS from two HV. Within-assay coefficients of variation (CVs) were 5.6 and 4.7%. The interassay CVs for both samples were 19.3 and 18.6%, respectively (5.19 ± 1.00 and 3.25 ± 0.60 —from ten different assays). Samples from a healthy individual have been used as internal control in every reaction. Our last collection of DBS control sample was assessed in 24 reactions during 11 months and the CV was 10.46%.

MPS I diagnosis was confirmed in 11 subjects by both assays and the enzyme activity from each one is described in Table 1. The delay between blood collection and arrival at our laboratory did not exceed 5 days. IDUA activity has been described to be stable for up to 21 days at room temperature and for 4 years at 4°C (7,12).

TABLE 1. DBS and Leukocyte Activities from Subjects Clinically Suspected of MPS I

Subjects	Gender	DBS activity	Leukocyte activity
1	Male	0.90	0.00
2	Female	2.03	9.09
3	Female	3.24	6.51
4	Female	7.80	8.99
5	#	3.16	19.25
6	Male	1.09	0.00
7	Female	0.95	0.00
8	Female	5.20	12.27
9	Female	20.05	15.28
10	Male	13.17	7.02
11	Female	13.06	8.55
12	Female	8.08	2.15
13	Male	0.57	0.02
14	Female	0.79	0.02
15	Female	3.67	30.88
16	Male	5.88	20.09
17	Male	0.51	0.00
18	Female	4.79	12.72
19	Male	3.78	1.15
20	Male	4.00	7.52
21	Male	0.62	0.02
22	Female	1.09	0.00
23	Female	6.66	21.12
24	Male	5.48	8.02
25	Female	0.49	0.00
26	Female	3.90	4.79
27	Male	6.99	20.45
28	Female	5.19	10.76
29	Male	4.31	8.28
30	Male	2.81	1.82
31	Male	3.44	0.77
32	Female	6.97	29.57
33	Female	3.92	9.57
34	Female	3.61	14.24
35	Male	0.98	0.00
36	Female	1.08	0.00

Subjects = Clinical suspected of MPS I. DBS activity is expressed as $\mu\text{mol/l blood/hr}$. Leukocyte activity is expressed as $\text{nmol/mg protein/hr}$. # = not informed. Shadowed rows indicate confirmed MPS I patients.

Fig. 1. IDUA activity on DBS samples from each subject in all experimental groups. HV group: healthy volunteers. MPS I negative: clinically suspected of MPS I subjects whose diagnoses were not confirmed by enzymatic assays. MPS I positive: clinical suspected of MPS I subjects whose diagnoses were confirmed by both enzymatic assays. Gray bars represent mean values. IDUA, α -L-iduronidase; DBS, dried blood spots; MPS, mucopolysaccharidosis type I.

Figure 1 shows DBS activities from all groups. There was no overlap among MPS I patients and HV activities (minimum activity from HV: 1.40; maximum activity from MPS I patients: 1.09). The residual enzyme activity of MPS I patients ranged from 0 to 33%, calculated as a percentage of the mean activity value from the HV group.

DISCUSSION

Results from the ROC curve suggested that a more appropriate cutoff value for IDUA activity on DBS samples would be 1.5 $\mu\text{mol/l}$ blood/hr, in which both sensitivity and specificity values were 100%. The positive and negative predictive values obtained were also 100%. With the cutoff of 1.5 $\mu\text{mol/l}$ blood/hr and considering 20% as the acceptable CVs for DBS assay (between 1.2 and 1.8 $\mu\text{mol/l}$ blood/hr) two HV were considered false positives (1.4 and 1.6 $\mu\text{mol/l}$ blood/hr). These two samples were reanalyzed and activity values remained among the same range.

A reference interval for IDUA activity in Brazilian HV was determined, and results were ranged between 1.40 and 7.78 $\mu\text{mol/l}$ blood/hr (11). No correlation was found between DBS and leukocytes activities, and as previously described by Chamoles and coworkers that difference is expected, since different types of cells and biological materials had been analyzed. In addition, those assays (though similar) show different sensitivity and specificity.

A ROC curve analysis from the VG enabled the establishment of the most appropriate cutoff value for Brazilian population, which was 1.5 $\mu\text{mol/l}$ blood/hr. With this new cutoff value, sensitivity, specificity, and predictive values were all maintained at 100%. The assay was sensitive enough to differentiate MPS I patients from healthy subjects, but two samples from the HV group (who did not show clinical signs or symptoms of MPS I) presented IDUA activity below 1.5. Thus, assuming that an interassay CV of up to 20% is acceptable for this type of method (7,13,14) IDUA activity values between 1.2 and 1.8 should be evaluated with caution, since they may represent activities from MPS I patients, activities from heterozygotes, or may be just a consequence of the expected variation of the assay.

Therefore, we suggest that the interpretation of IDUA activity results should be performed according to the following criteria: (1) for activity results lower than 1.2 $\mu\text{mol/l}$ blood/hr, the DBS method is an accurate tool to diagnose MPS I, together with the evaluation of clinical symptoms and other laboratorial results; (2) for activity results between 1.2 and 1.8 $\mu\text{mol/l}$ blood/hr, another diagnostic assay should be performed in leukocyte, plasma, or fibroblast samples to confirm these suggestive results; and (3) for activity results higher than 1.8 $\mu\text{mol/l}$ blood/hr, the DBS assay is reliable and safe to exclude the diagnosis of MPS I.

Once the stability and practicality of this material allow safe and fast transportation (including mailing in regular envelopes) the use of DBS samples for diagnosis of MPS I patients could be very useful in countries or regions with a large extension like Brazil, and mainly where there are few reference laboratories performing this type of enzymatic assays. It has been observed that samples sent by regular mail maintain enzyme activity for at least 1 month (data not shown), which corroborates with literature data (7,12,13).

In addition, the use of DBS minimizes biological hazards, and only a small amount of blood is required for such diagnosis. Around 30 samples can be analyzed simultaneously by this microplate assay, which reduces further costs and may anticipate the communication of test results to the physicians.

It is also clear that each center should develop its own IDUA activity cutoff value based on the characteristics of the population, which will be evaluated, and on specific laboratory/assay conditions. This reference cutoff should take age into account, since it is reported that newborns have a different range of enzyme activity values (7,14), but a gender-specific reference value is not necessary, since no significant difference in IDUA activity between males and females has been observed (11).

Our results suggest that the determination of IDUA activity using a DBS assay is an accurate tool for MPS I

diagnosis. However, it is extremely important to assure that a correct blood collection and DBS preparation is performed, followed by safe transportation and adequate storage, to guarantee the quality of the samples. It is also important to emphasize that in vitro assay results should always be analyzed together with other information as clinical symptoms and familial history.

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